Summary. Within 10-20 days after complete hypophysectomy the B.M.R. of the adult pigeon undergoes a pronounced decrease, but the extent of the indicated decrease is much influenced by the environmental temperature at which measurement is made. At the critical temperature (30°C.) the B.M.R. of 9 pigeons was decreased by 33%; in 7 pigeons measured at 20°C. the decrease was only half as much, or 17%. Seven incompletely hypophysectomized birds (less than 4% of pituitary present) measured at 30° provide data which confirm the important rôle of temperature and further indicate that the B.M.R. of such birds is practically the same as that of the completely hypophysectomized pigeon. Pituitary fragments representing 10-25% of the total gland usually only partially sustain the normal rate of heat production. B.M.R. measurements made at 20° did not distinguish partial from complete hypophysectomies, and this provides a further indication that a truer measure of effect of hypophysectomy is obtained from tests made at the animal's critical temperature.

8200 P

Heparin as an Anticoagulant in the Brucella Phagocytic Index Test.

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In a previous publication,[1] the application of the Brucella phagocytic index test to the study of epidemiology of undulant fever was discussed. Tests carried out on over 1,000 people seemed to indicate that the test was of value in the detection of passed, latent and present infections with the Brucella organisms.

When the same technic was applied to the natural hosts, goats and cattle, the differences between the normal and the infected animals were not striking. Although positive indices were obtained in some of the smaller laboratory rodents, in larger animals the phagocytic activity of the leucocytes, despite the presence of Brucella organisms in the tissues, was either absent or very low. The indices of the infected or immunized animals were, however, consistently high, when, instead of whole citrated blood, the sedimented cells thorough-

[1] Meyer, Stewart, Veazie, and Eddie, PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 284.

ly washed and resuspended in Locke's gelatin solution were mixed with fresh serum and a bacterial suspension in equal quantities. From these observations it was concluded that the citrate solution was toxic for the leucocytes of certain herbivorous animals or inhibited in some way the phagocytic activity of the granulocytes. Since the washed cell method was too time-consuming for routine use, it was decided to search for an anticoagulant that would be more suitable for the purpose. Several were tried, including sodium oxalate, sodium fluoride, "Liquoide", amniotic fluid, and heparin. Heparin* was found to be far the most effective. A 1-1,000 dilution of heparin in saline (1 drop of blood to 1 drop of heparin solution) for human blood and 0.2 cc. of a 1:200 dilution for cattle and goats was employed, the test being conducted otherwise exactly as with citrate.

In Table I the significant data are summarized. A comparison of the indices determined on the leucocytes of citrated and heparinized blood clearly indicates that cattle as well as goats yield much higher indices when heparin is employed as an anticoagulant. In a group of recently infected cows the average index of the granulocytes in citrated blood was 1.6 and in heparin-treated blood it was 13.6. A herd with a record of freedom from abortion disease for over 6 years yielded one animal with an index above 5 with heparinized cells. Blood specimens of goats revealed the same differences. In fact, the existence of an infection was, as a rule, reflected by the index figure provided the blood specimen had been treated with heparin. An experimental group of goats infected and in various stages of latent Malta fever had always furnished specimens with negative phagocytic indices with citrate but showed highly active leucocytes and indices from 1 to 18 in the heparinized blood.

In a series of 100 human beings with active or latent Brucella infections, in which the phagocytic tests were carried out in duplicate, using both citrate and heparin, the differences between the 2 anticoagulants were not so apparent, though the average index in the heparin series was somewhat higher, being 5.8 as against 4.3 for citrate. Furthermore, in rare instances the blood of a febrile patient with a negative history for undulant fever gave with heparin an index figure suggestive of a latent infection. During convalescence and after recovery the index invariably dropped to the level typical for a normal human being.

* Since this anticoagulant had been tested experimentally for over 8 months, Boerner and Mudd, *Am. J. Med. Sc.*, 1935, **189**, 23, independently recommended its use.

TABLE I.
Comparative Phagocytic Indices in Blood Specimens Treated with Heparin and Sodium Citrate.

	Serologically (agglutination and complement fixation) and allergically positive					Serologically and allergically negative				
Species	No. tested	Aver. heparin index	Range	Aver. citrate index	Range	No. tested	Aver. heparin index	Range	Aver. citrate index	Range
Goats	27	7.0	1.8-18.6	0.7	0 - 2.7	21	1.1	0-4.3	0.3	0-0.5
Cattle	10	13.6	7.5-21.8	1.6	0 -10.8	30	3.0	0-5.08	0.07	0-1.0
Guinea pigs	8	13.6	4.3-27.6	4.8	0.4- 8.4	—	—	—	—	—
Humans	100	5.8	0 -27.3	4.3	0 -24.0	—	—	—	—	—
Incubation										
30 min.	12	8.4	0 -27.3	3.4	0 -11.6					
60 '	12	13.4	0 -35.9	5.5	0 -19.4					

It should be stressed that the inaccuracies inherent to the method are numerous, and too great importance should not be placed on minor variations in the index. Although experience has taught that false positives are quite uncommon, a negative result should not be accepted as final until 2 or 3 specimens of blood have been taken at varying intervals. A trace of acid in a tube, too long an interval between the collection of the blood and the addition of the organisms, a slight alteration in the pH of the reagents, partial clotting of the blood, or the admixture of tissue juice when the blood is secured by puncture of the ear or finger instead of the vein may lower or entirely prevent phagocytosis of the Brucella organisms. The length of the incubation period at 37°C. alters the index markedly in many instances, the number of bacteria ingested being often twice as great at the end of an hour as after 30 minutes. Whether there is a difference in the susceptibility of leucocytes to external influences which may change from day to day is not known, but it has been noted that blood taken from the same person on different days, though the conditions of the test were as nearly identical as possible, gave widely differing results. It is recommended that when possible, tests be run in duplicate, using both heparin and citrate as anticoagulants, until the factors controlling the phenomenon are more fully understood.

8201 P

Action of Carbaminoylcholine on the Iris, Normal and Parasympathectomized.

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The ciliary ganglion and its accessories were removed from one orbit in each of a series of cats. At intervals thereafter, the effects on the pupils of a drop of carbaminoylcholine chloride solution in each eye, were studied in the dark-room under conditions of uniform lighting. In the experiments here reported, the dosage in each eye was equivalent to 0.3 mg. of carbaminoylcholine chloride and the eyes, when studied, were 60 cm. from a screen of white muslin, illuminated by a 100 watt mazda lamp, 40 cm. away.

When the irises were tested *within 24 days* after the operation,